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The Influence of Crude Protein Level in the Basal Diet on the Determination of Lysine Requirements for Broiler Performance and Part Yields

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ABSTRACT

Amino acid requirements can be studied maintaining or not the ratio of the amino acids to lysine and changing or not dietary crude protein level. A third alternative was studied in this study conducted to evaluate broiler performance and weight gain of carcass parts, in response to increasing dietary digestible lysine (dig Lys) levels (0.70, 0.80, 0.90, 1.00, 1.055, 1.11, 1.165, and 1.22%). Two basal diets were formulated to contain 19.0% (diet A) and 20.5% (diet B) crude protein, and Met, Arg and Thr levels were formulated to maintain their ideal ratio to Lys. Three hundred and twenty CobbXCobb500 broilers, from 19 to 40 days of age, were used. Basal diet A was set for the 4 lowest levels of dig Lys, and diet B for the other four levels. Body weight (BW), weight gain (WG), feed intake, Lys intake, feed conversion (FC), carcass part weights and carcass, breast, leg, and thigh protein and fat were evaluated. Body weight, WG and Lys intake linearly increased with increasing dietary dig Lys, independently of basal diet CP content. A multiple regression effect was observed for FC, with the best estimated levels of dig Lys of 0.96% and 1.18% for diet A and B, respectively. Breast and carcass weight gain and breast protein and water gain showed multiple regression and split curves as a function of basal diet. The best responses were obtained with the highest crude protein level in the basal diet. Therefore, we concluded that high levels of crude protein in basal diets are recommended to study amino acid requirements for broilers.

INTRODUCTION

Lysine is considered the second limiting amino acid in broiler diets. It is used as the reference amino acid in poultry and swine nutrition because it is mainly utilized for protein synthesis (D'Mello, 2003) and accounts for 7.5% of carcass protein (Sklan & Noy, 2004).

The most representative body compartment of the body amino acid pool is the skeletal muscle (Fernández-Fígares *et al.*, 1997). As the breast muscle accounts for 60% of the edible protein of the broiler carcass (Labadan *et al.*, 2001), it is an important component when studying animal response to dietary essential amino acid and total protein levels.

Amino acid requirements are influenced by several environmental (Borges *et al.*, 2002), genetic (Fatufe *et al.*, 2004), and nutritional (Urdaneta-Rincon *et al.*, 2005) factors. In this context, it was shown that modern broiler strains, with high potential for the accretion of valuable parts, such as the muscle, have high dietary lysine requirements (Fatufe *et al.*, 2004).

Studies carried out to determine lysine requirements may change lysine dietary level, while maintaining dietary crude protein content (Borges *et al.*, 2002), and may consider or not lysine ratios to the other dietary amino acids (Lana *et al.*, 2005). However, ratios among amino acids should be maintained when evaluating responses to lysine supplementation levels, as the imbalance of other amino acids to lysine may negatively influence the assessed parameters (Lana *et al.*, 2005).

Another method used to determine lysine requirements is to change dietary lysine levels while maintaining its ratio to the other amino acids, but proportionally changing dietary crude protein content (Moran *et al.*, 1992). According to Sterling *et al.* (2003), for each crude protein level, there is an optimal lysine level for the evaluated parameter. Therefore, dietary crude protein level may influence the determination of optimal amino acid content in diets (Urdaneta-Rincon *et al.*, 2005).

The present study aimed at determining lysine requirements for performance, protein accretion, fat deposition, water deposition, and carcass yield, as well as parts yield (breast and legs) of 19- to 40-day-old broilers. Lysine levels were distributed in two crude protein levels, maintaining amino acid balance, according to Rostagno *et al.* (2005). Therefore, two basal diets were used: one with 19.0 and the other with 20.5% crude protein.

MATERIAL AND METHODS

A number of 320 male CobbXCobb500 broilers was distributed in a completely randomized experimental design with a 2 x 4 factorial arrangement (protein and lysine levels, totaling eight treatments with five replicates of eight birds each. At 19 days of age, birds were housed in an environmentally-controlled room containing 40 0.72-m^2 cages. Room temperature was set within the daily thermal comfort range. Water was offered ad libitum. Until 19 days of age, birds were fed mash diets, formulated to supply their nutritional requirements (Rostagno *et al.*, 2005).

Between 19 and 40 days of age, eight experimental diets were fed. Treatments consisted of two basal diets with graded levels of digestible lysine (dig. Lys - 0.70, 0.80, 0.90, 1.00, 1.055, 1.11, 1.165, and 1.22 %). The four lowest dig. Lys levels were added to basal diet A, containing 19.0% crude protein (CP), and the remaining four were added to basal diet B, which contained 20.5% CP. The diets, based on corn, soybean meal, and corn gluten, were fed in the mash form, and formulated to maintain minimum ratios of the amino acids (AA) methionine (Met), threonine (Thr), and arginine (Arg) to lysine, according to Rostagno *et al.* (2005) (Table 1). The objective of using two basal diets was to maintain amino acid balance, precluding the need of excessive synthetic amino acid supplementation in the treatments with higher lysine levels, thereby preventing the interference of synthetic amino acid sources on diet digestibility.

Bird average body weight (BW), weight gain (WG), feed intake (FI) and feed conversion ratio (FCR) were determined when birds were 40 days of age. At 19 days of age, five birds were sacrificed to determine their body composition, which was used as reference of the beginning of the experimental period. At 40 days of age, two birds per cage with body weight similar to the cage average weight were selected, according to the method of Corzo *et al.* (2003), and sacrificed. Their carcasses were cut into the following parts: thighs+drumsticks (leg), breast, and carcass. The carcass consisted of bird weight minus feathers, offal, and blood. Absolute weight gain of the body components was determined for the period of 19 to 40 days of age. Dry matter content from each part was determined at 105ºC, and their water content was determined as the difference. Protein and fat content were determined in the laboratory according to the AOAC (1996).

Data were submitted to statistical analysis using SAS (2001) software package for linear, quadratic, and multiple regressions with a single intercept in a split-plot model, from which the best fit were selected according to the evaluated response (>r^2) and significance of the parameters (coefficients) b_0, b_1, b_2, b_3, b_4. The following models were applied:

Linear: $Y = b_0 + b_1*lys$;
Quadratic: $Y = b_0 + b_1*lys + b_2*lys_2$;
Multiple: $Y = b_0 + b_1*lysA + b_2*lysA_2 + b_3*lysB + b_4*lysB_2$,

where: Y = evaluated parameter; b_0 = common intercept of the basal diets; b_1, b_2, b_3, b_4 = coefficients of linear regression, with b_1 and $b_2 = 0$ when lys > 1, and b_3 and $b_4 = 0$ when lys ≤ 1.0.

RESULTS AND DISCUSSION

Table 2 shows the analyses of regression relative to bird performance. The best fit of the equations (>R2) was obtained with linear regressions for the parameters BW, WG, and lysine intake (LysI), which values increased as dietary dig. Lys supplementation increased, independently from CP content in the basal

Table 1. Ingredients and nutritional levels of the experimental diets.

Ingredients (%)	Dig. Lys (%)							
	Basal A (19% CP)				Basal B (20.5% CP)			
	0.7	0.8	0.9	1.0	1.055	1.11	1.165	1.22
Corn[1]	64.00	64.00	64.00	64.00	53.85	53.85	53.85	53.85
SBM 45%[1]	20.08	20.08	20.08	20.08	34.91	34.91	34.91	34.91
Corn gluten 60%[1]	9.47	9.47	9.47	9.47	2.00	2.00	2.00	2.00
Vegetable oil	1.90	1.90	1.90	1.90	4.84	4.84	4.84	4.84
Dicalcium phosphate	1.77	1.77	1.77	1.77	1.73	1.73	1.73	1.73
Limestone	1.18	1.18	1.18	1.18	1.06	1.06	1.06	1.06
Starch	0.80	0.64	0.46	0.10	0.61	0.45	0.28	0.07
Salt	0.35	0.31	0.27	0.23	0.45	0.45	0.42	0.40
Na bicarbonate	0.14	0.20	0.26	0.32	-	0.03	0.06	0.10
Choline	0.11	0.11	0.11	0.11	0.04	0.04	0.04	0.04
Monensin 20%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Min Premix *	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vit Premix **	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
L-Lysine, HCl[2]	-	0.13	0.26	0.38	0.11	0.18	0.25	0.32
DL-Methionine	-	0.01	0.05	0.12	0.17	0.21	0.25	0.29
L-Threonine	-	-	-	0.04	0.01	0.04	0.08	0.11
L-Arginine	-	-	-	0.10	-	-	0.02	0.08
Calculated nutrients								
ME, kcal/kg	3100	3100	3100	3099	3106	3106	3106	3105
CP, %	19.00	19.15	19.33	19.76	20.50	20.64	20.81	21.06
Ca, %	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Avail. P, %	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Na, %	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline, mg/kg	1400	1400	1400	1400	1400	1400	1400	1400
Na+K-Cl, meq/kg	150	150	150	150	190	190	190	190
Monensin, mg/kg	100	100	100	100	100	100	100	100
Dig. Lys, %[3]	0.70	0.80	0.90	1.00	1.055	1.11	1.17	1.22
Dig. Met+Cys, %[3]	0.60	0.61	0.65	0.72	0.76	0.80	0.84	0.88
Dig. Met, %[3]	0.31	0.32	0.36	0.43	0.45	0.49	0.53	0.57
Dig. Arg, %[3]	0.95	0.95	0.95	1.05	1.20	1.20	1.22	1.28
Dig. Thr, %[3]	0.62	0.62	0.62	0.66	0.69	0.72	0.76	0.79

1 - Ingredients analyzed for total AA content (Ajinomoto Biolatina Ind. e Com. Ltda em São Paulo). 2 - L-Lysine monochloride, with 99% purity, AjiLys® from Ajinomoto Biolatina Ind. e Com. Ltda. 3 - Digestible amino acid levels were calculated using the coefficients of amino acid digestibility for each ingredient, according to (2005).*Mineral premix: 0.3 mg Se, 0.8 mg I, 25 mg Fe, 9 mg Cu, 60 mg Zn, and 70 mg Mn per kg diet. **Vitamin premix: 7000 IU vit. A, 1500 IU vit. D3, 25 mg vit. E, 3.5 mg vit. K3, 1 mg vit. B1, 4 mg vit. B2, 1.6 mg vit. B6, 10 mcg vit. B12, 9 mg pantothenic acid, 25 mg niacin, 0, 5 mg folic acid, and 20 mcg biotin per kg diet.

Table 2. Regression equations and optimal digestible lysine levels obtained for the performance parameters determined in broilers fed two different basal diets between 19 and 40 days of age.

Parameter(g)	Regression model	EquationProb.		r^2	Optimal Lys 19.0 / 20.5
BW[1]	Linear	Y=2016+807lis	<0.0001	0.603	≥1.22
WG[2]	Linear	Y=1158+802lis	<0.0001	0.599	≥1.22
FI[3]			NS		
LysI[4]	Linear	Y=-0.592+34.3lis	<0.0001	0.930	
FCR[5]	Multiple	$Y=4.27-5.20lisA+2.71lisA2-4.53lisB+1.93lisB^2$	<0.0001	0.897	0.96 / 1.18

1 - Average body weight; 2 - Weight gain; 3 - Feed intake; 4 - Lysine intake; 5 - Calculated feed conversion ratio.

diet. Only FCR presented the best fit using multiple regression (Figure 1). Linear regression also presented the best fit for parts (Table 3) (weight gain, leg protein and water gain, and carcass protein and water gain), as well as for fat deposition in all parts. Multiple regression best explained breast and carcass weight gain, and breast protein and water gains.

Parameters where multiple regression presented the best fit show that the 1.5% difference in CP content between the basal diets influenced the response curves, indicating a split in these curves. This resulted in two different optimal dig. Lys levels - one for each basal diet (Tables 2 and 3). As to FCR, for instance, the estimated optimal dig. Lys level was 0.96% for basal diet A, and 1.18% for basal diet B (Table 2 and Figure 1). This phenomenon was statistically significant in the cases of FCR, breast and carcass weight gain, and breast protein and water weight. This shows that the breast was the part most affected by dietary CP differences. It must be mentioned that breast weight considerably influences carcass weight, which may explain the effect also on carcass weight. However, when carcass protein and water are separately analyzed, this effect disappears. In the case of breast protein gain, optimal dig. Lys level was 0.91% for basal diet A, and 1.13% for basal diet B, as shown in Figure 2.

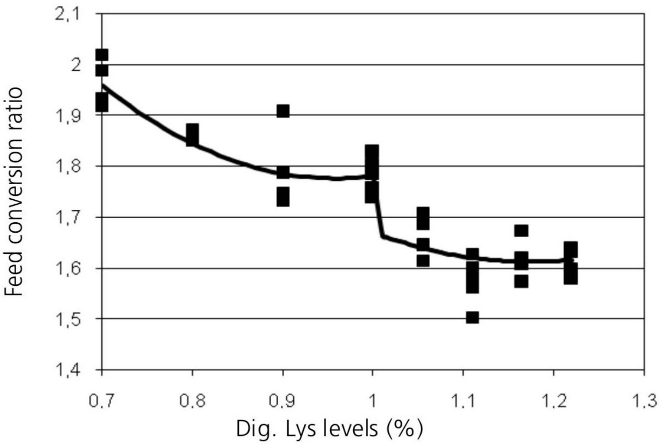

Figure 1. Feed conversion ratio of 19- to 40-day-old broilers fed diets with graded levels of digestible lysine distributed in two basal diets with different crude protein contents.

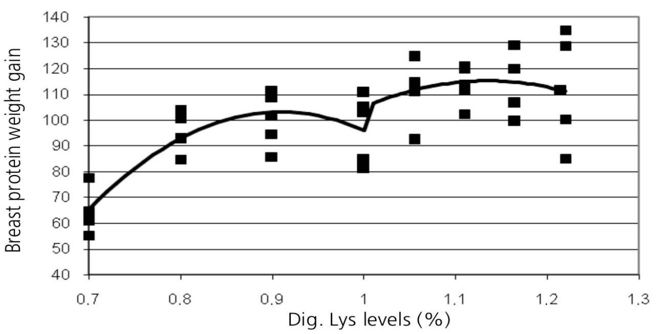

Figure 2. Breast protein gain of 19- to 40-day-old broilers fed diets with graded levels of digestible lysine distributed in two basal diets with different crude protein contents.

Sterling *et al.* (2003) found different optimal lysine levels for weight gain that changed according to dietary crude protein level. Therefore, total dietary crude protein level changes result in different response curves for increasing levels of limiting amino acids (D'Mello, 2003). Urdaneta-Rincon & Leeson (2004) verified weight gain increase in broilers fed graded lysine levels in diets with CP contents between 170 and 290 g/kg. The estimated optimal lysine level was higher than 1.22%, independently from dietary CP. In the study of Sterling *et al.* (2003), the Best FCR values were obtained when basal diet CP content was increased from 17% to 23%, using 0.97% dig. Lys. According to Urdaneta-Rincon *et al.* (2005), the higher the protein content in the diet, the higher the lysine level required, and the higher the nitrogen retention. Lana *et al.* (2005) found a linear FCR improvement when diets were supplemented with lysine; this effect was more evident in amino-acid balanced diets as compared to non-balanced feeds.

According to Fernández-Fígares *et al.* (1997), differences in amino acid requirements may result from different dietary crude protein levels, amounts of added synthetic AA, and CP quality in the basal diet, interfering in the determination of amino acid requirements. According to Sterling *et al.* (2006), broiler strains with higher weight gain potential, such as Ross and Cobb, suffer a stronger influence of dietary CP content than other genetic strains.

The r^2 values of the linear equations were not very different from those obtained in the multiple regression curves. For FCR, which presented the higher similitude among regressions, the r^2 of the linear regression was 0.827, whereas 0.897 was obtained with the multiple regression equation. This explains why the estimates of dig. Lys requirements are lower for FCR than for WG (1.18 *vs.* ≥ 1.22%), when the opposite is usually observed. The lower variability in FCR allowed us to obtain a significant curve with the multiple regression equation, which was not the case with WG, better explained by the linear model. For breast weight, the r2 values were 0.481 for the linear and 0.642 for the multiple regression models; for carcass weight, 0.473 for linear and 0.607 for multiple regression; for breast protein gain, 0.586 for linear and 0.674 for multiple regression; for breast water gain, 0.547 for linear and 0.606 for multiple regression. The results indicate that breast weight, protein, and water, and carcass weight are more influenced by CP levels in the basal diet than FCR.

The effect of splitting as a function of basal diet was not detected for BW; WG; leg protein and water gains, carcass protein and water gains, or fat gain in the different parts. The linear effect shows that lysine level itself is determinant, with no interference of basal diet crude protein levels. In addition, these responses show that birds respond to digestible lysine levels higher than those recommended by Rostagno *et al.* (2005).

There was no significant effect of digestible lysine levels on feed intake (Table 2). This response is consistent with the findings of Lana *et al.* (2005), using

Table 3. Regression equations and optimal digestible lysine levels obtained for weight and nutrient gain in the body parts of broilers fed two different basal diets between 19 and 40 days of age.

Gain	Part	Regression model	Equation	Prob.	r^2	Optimal Lys level 19/20.5
Weight	Breast	Multiple	$Y=-2230+5991lysA-3317lysA^2+4745lysB-2060lysB^2$	<0.0001	0.642	0.90 / 1.15
	Leg	Linear	Y=305+188lys	0.0002	0.315	≥1.22
	Carc.	Multiple				
			$Y=-2527+9092lysA-5174lysA^2+6957lysB-2920lysB^2$	<0.0001	0.607	0.88 / 1.19
Protein	Breast	Multiple	$Y=-612+1573lysA-865lysA^2+1282lysB-565lysB^2$	<0.0001	0.674	0.91 / 1.13
	Leg	Quadr.	$Y=165-228lys+146lys^2$	0.0258	0.496	≥1.22
	Carc.	Linear	Y=97.4+181lys	<0.0001	0.590	≥1.22
Water	Breast	Multiple	$Y=-1146+3120lysA-1674lysA^2+2448lysB-1001lysB^2$	<0.0001	0.606	0.93 / 1.22
	Leg	Quadr.	$Y=350-420lys+249lys^2$	0.0661	0.256	≥1.22
	Carc.	Linear	Y=271+659lys	<0.0001	0.631	≥1.22
Fat	Breast	Linear	Y=54.0-13.2lys	0.0225	0.130	≥1.22
	Leg	Linear	Y=103-28.1lys	0.0031	0.208	≥1.22
	Carc.	Linear	Y=317-98.8lys	0.0006	0.271	≥1.22

Carc.: Carcass.

a single crude protein level, and of Sterling *et al.* (2006), varying CP levels fed to different broiler strains. On the other hand, Sterling *et al.* (2003) observed an effect on feed intake of lysine supplementation levels within the same CP content, when AA dietary ratio was maintained. However, there was no difference in feed intake when CP in the basal diet was increased from 17% to 23%. Urdaneta-Rincon & Leeson (2004) found segregation of breast muscle deposition curves as a function of CP content in the basal diet: diets containing higher CP level (250g/kg) promoted higher breast muscle deposition at 1.3% lysine as compared to a lower CP content (210g/kg). As to commercial parts and abdominal fat weights, Sterling *et al.* (2006) did not detect any influence of dietary CP level (17 or 23%).

CONCLUSIONS

In studies performed to determine amino acid requirements of broilers, basal diet protein content influences the type of response and the optimal levels of the evaluated amino acids. For FCR and breast weight gain, the optimal levels of digestible lysine were 0.96 and 1.18%, and 0.90 and 1.15%, respectively, for basal diet with 19% and 20.5% CP. When evaluating breast responses, higher protein levels in the basal diets are suggested, as the use of basal diets with low protein content may underestimate practical lysine requirements. The increasing performance responses promoted by increasing digestible lysine levels suggest that the dietary levels of this amino acid are higher than those currently recommended for the Cobb500 strain. However, the economics of free amino acid supplementation to diets must be taken into account.

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